**Texas A & M University**

**Principal Investigator:** Kevin Burgess
**Co-Investigators:** Fred Schroeder and Robin Hochstrausser

**Fluorescent Probes for Multiplexed Intracellular Imaging**

The focus of our center is to design, prepare, and investigate novel fluorescent probes, termed "through-bond energy transfer cassettes", for intracellular imaging. At this stage, it involves chemical syntheses of the probes (Burgess), conjugation to proteins involved in a signaling cascade for nuclear localization, and observation in cells, both under conventional conditions (Schroeder) and on a single-molecule level (Hochstrasser). Throughout, proteins labeled with the cassettes are transported into the cells using the carrier peptide known as Pep-1 or Chariot™.

Kevin Burgess' group has prepared a range of donor and acceptor fragments for assembly into through-bond energy transfer cassettes. These include functionalized derivatives of fluorescein and Nile Red (which atypically have high quantum yields in aqueous solution), novel GFP fluorescent site analogs, extended BODIPY dyes, aza-BODIPY, and rhodamines. Some of these have been assembled into cassettes, and work is in progress to make more. Fluorescent properties of the cassettes show some "leakage" of energy through the donor part, which decreases on addition of Triton X100. We believe that even thought the cassettes are somewhat soluble in water, they may still aggregate, thus causing this effect. The current emphasis of the research is to increase water solubility as a means to decrease aggregation. Our group has also designed a novel chemiluminescent cassette.

Fred Schroeder has purified recombinant ACBP, L-FABP and HNF-4a, has labeled these compounds with fluorescent dyes and cassettes for uptake into cells, and has determined their multi-protein interactions. While ACBP and HNF-4a interact *ex vivo* and in cells, new evidence indicates that L-FABP also interacts with HNF-4a. To ensure that the structural and functional properties of these proteins are maintained after dye conjugation, CD, MALDI-MS, fluorescence binding, FRET assays, as well as LSCM colocalization in live cells were performed. Results demonstrated the following: (i) newly synthesized water-soluble fluorescent dyes and cassettes allow greater protection of structural and functional properties of ACBP as compared to Cy3 or Cy5; (ii) L-FABP and HNF-4a, which unlike ACBP have a high content of β-strands, remain largely unchanged in secondary structure composition and ligand affinity, more so than ACBP. The mechanism of Pep-1-mediated protein uptake was studied by estimating the colocalization of fluorescent-labeled ACBP with membrane domains and vesicles probed by fluorescent sterols (dansyl-cholesterol, DHE). The effect of Pep-1 on the intracellular location of dye-ACBP was determined by colocalization of membrane-organelle markers with fluorescent-labeled ACBP.

Robin Hochstrasser and co-workers have investigated the interaction of Pep-1 with model lipid membranes by fluorescence imaging at the single-molecule level. Association of Pep-1, labeled either on the C- or N-terminus, with the surface of giant unilamellar vesicles was studied at different membrane charge and solution ionic strength. Pep-1 interacts mainly due to electrostatic rather than hydrophobic forces. It induces alterations of structure of the giant vesicles and promotes fusion and sedimentation of large unilamellar vesicles. Pep-1 does not

induce adsorption of five mid-size globular proteins to the bilayer. Experiments of translocation of Pep-1/ACBP in vivo in single COS7 cells did not provide evidence for any significant effect of Pep-1. Thus, the translocation efficiency of Pep-1 may be related to cell confluency. The binding of labeled ACBP protein to the lipid membrane allowed tracking experiments to reveal a large heterogeneity in bilayer fluidity. A new method of wide-field subdiffraction imaging has been developed and is being used to examine the binding of RBCI-ACBP protein to supported bilayers at 20 nm resolution.

**Publications**


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